abcam

Product datasheet

Human MSH6 knockout HeLa cell line ab255410

画像数 3

製品の概要

製品名	Human MSH6 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Component of the post-replicative DNA mismatch repair system (MMR). Heterodimerizes with MSH2 to form MutS alpha, which binds to DNA mismatches thereby initiating DNA repair. When bound, MutS alpha bends the DNA helix and shields approximately 20 base pairs, and recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. After mismatch binding, forms a ternary complex with the MutL alpha heterodimer, which is thought to be responsible for directing the downstream MMR events, including strand discrimination, excision, and resynthesis. ATP binding and hydrolysis play a pivotal role in mismatch repair functions. The ATPase activity associated with MutS alpha regulates binding similar to a molecular switch: mismatched DNA provokes ADP-->ATP exchange, resulting in a discernible conformational transition that converts MutS alpha into a sliding clamp capable of hydrolysis-independent diffusion along the DNA backbone. This transition is crucial for mismatch repair. MutS alpha may also play a role in DNA homologous recombination repair.
関連疾患	Defects in MSH6 are the cause of hereditary non-polyposis colorectal cancer type 5 (HNPCC5) [MIM:600678]. Mutations in more than one gene locus can be involved alone or in combination in the production of the HNPCC phenotype (also called Lynch syndrome). Most families with clinically recognized HNPCC have mutations in either MLH1 or MSH2 genes. HNPCC is an autosomal, dominantly inherited disease associated with marked increase in cancer susceptibility. It is characterized by a familial predisposition to early onset colorectal carcinoma (CRC) and extra-colonic cancers of the gastrointestinal, urological and female reproductive tracts. HNPCC is reported to be the most common form of inherited colorectal cancer in the Western world. Cancers in HNPCC originate within benign neoplastic polyps termed adenomas. Clinically, HNPCC is often divided into two subgroups. Type I: hereditary predisposition to colorectal cancer, a young age of onset, and carcinoma observed in the proximal colon. Type II: patients

have an increased risk for cancers in certain tissues such as the uterus, ovary, breast, stomach, small intestine, skin, and larynx in addition to the colon. Diagnosis of classical HNPCC is based on the Amsterdam criteria: 3 or more relatives affected by colorectal cancer, one a first degree relative of the other two; 2 or more generation affected; 1 or more colorectal cancers presenting before 50 years of age; exclusion of hereditary polyposis syndromes. MSH6 mutations appear to be associated with atypical HNPCC and in particular with development of endometrial carcinoma or atypical endometrial hyperplasia, the presumed precursor of endometrial cancer. Defects in MSH6 are also found in familial colorectal cancers (suspected or incomplete HNPCC) that do not fulfill the Amsterdam criteria for HNPCC.
Defects in MSH6 are a cause of susceptibility to endometrial cancer (ENDMC) [MIM:608089].

配列類似性 Belongs to the DNA mismatch repair mutS family.
Contains 1 PWWP domain.

翻訳後修飾 The N-terminus is blocked.
Phosphorylated upon DNA damage, probably by ATM or ATR.
Phosphorylated by PRKCZ, which may prevent MutS alpha degradation by the ubiquitin-proteasome pathway.

細胞内局在 Nucleus.

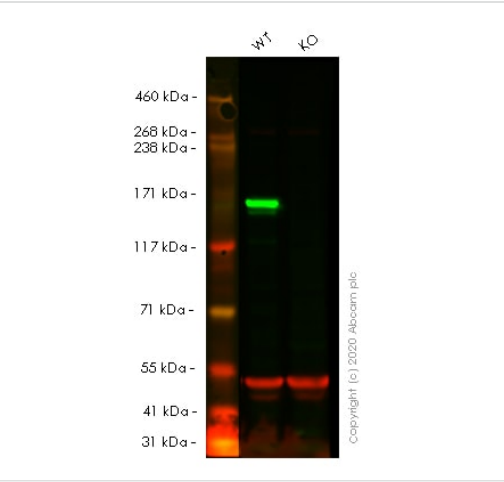
アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab255410の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 153 kDa.

画像



Western blot - Human MSH6 knockout HeLa cell line (ab255410)

All lanes : Anti-MSH6 antibody [44] (**ab14204**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MSH6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

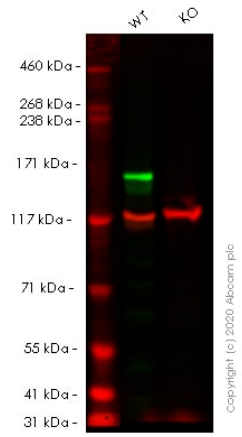
Performed under reducing conditions.

Predicted band size: 153 kDa
Observed band size: 160 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab14204**

observed at 160 kDa. Red - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) observed at 50 kDa.

ab14204 was shown to react with MSH6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255410 (knockout cell lysate **ab263763**) was used. Wild-type HeLa and MSH6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab14204** and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (**ab52866**) overnight at 4°C at a 1 in 500 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye®800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye®680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human MSH6 knockout HeLa cell line (ab255410)

All lanes : Anti-MSH6 antibody [EPR3945] (**ab92471**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : MSH6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 153 kDa
Observed band size: 160 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab92471** observed at 160 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab92471 was shown to react with MSH6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255410 (knockout cell lysate **ab263763**) was used. Wild-type HeLa and MSH6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab92471** and Anti-Vinculin antibody [VIN-54] overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L

(IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human MSH6 knockout HeLa cell line (ab255410)

Homozygous: 1 bp insertion in exon 4.

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